

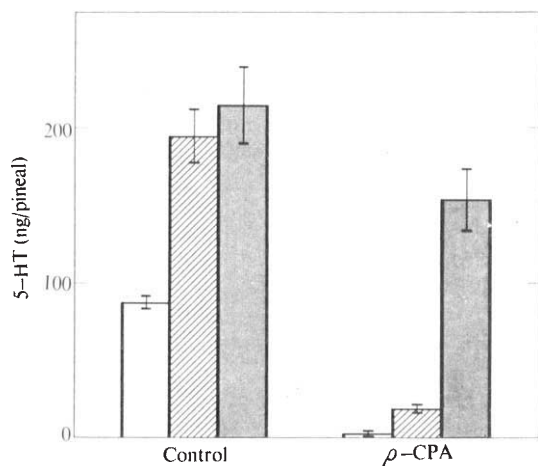


Fig. 1 Serotonin in the pineal after injection of L-tryptophan. L-Tryptophan (20 mg, or 500 mg/kg of body weight) was injected intraperitoneally to rats. After 30 min rats were decapitated and serotonin was analysed. Each group consists of ten to twenty rats. Two pineals were pooled for one analysis. Vertical bars indicate standard errors of the mean. White columns, no L-tryptophan; hatched columns, 20 mg/kg; tinted columns, 500 mg/kg.

*et al.*⁶ using a 'Bio-Rex 70' (Na⁺ form, 200 to 400 mesh) column (0.4 × 2.0 cm).

Tryptophan hydroxylase activity in the pineal of *p*CPA-treated rats was 60% of that in control rats, when assayed at a concentration of 10⁻⁵ M L-tryptophan (Table 1). When assayed at a concentration of 10⁻⁴ M L-tryptophan, tryptophan hydroxylase activity reached the same value in the pineals of both control and *p*CPA treated rats. The result indicates that *p*CPA does not inactivate tryptophan hydroxylase in rat pineals. Tryptophan hydroxylase, however, might be competitively inhibited by treatment with *p*CPA. The inhibition was overcome by increasing the concentration of L-tryptophan in the assay mixture. Tryptophan hydroxylase activity in the brain, on the other hand, was less than 5% of that in control rats in the conditions used here.

*p*CPA depletes serotonin level in rat pineal⁷, posing the question whether or not the pineals of *p*CPA-treated rats can synthesize serotonin from tryptophan *in vivo*. L-Tryptophan was therefore injected intraperitoneally into rats, and serotonin in the pineal was measured. Serotonin in the pineals of control rats increased three-fold after administration of L-tryptophan in a dose of 500 mg/kg (Fig. 1). With 20 mg/kg of L-tryptophan, serotonin in the pineal of control rats was almost the same as that with 500 mg/kg of L-tryptophan, indicating that tryptophan hydroxylase in the pineal is almost saturated with substrate at a dose of 20 mg/kg of L-tryptophan. On the other hand, the serotonin in the pineal of *p*CPA-treated rats was negligible. At a dose of 20 mg/kg serotonin slightly increased. At a dose of 500 mg/kg, however, serotonin increased by 150 ng/pineal, which is almost the same increase observed in the pineal of control rats.

The results obtained *in vivo* and *in vitro* show that *p*CPA does not inactivate tryptophan hydroxylase in the pineal of rats, but does lower the affinity of tryptophan hydroxylase for the substrate, presumably by competitive inhibition. In normal conditions, the pineal of *p*CPA-treated rats cannot synthesize serotonin because the *K_m* of tryptophan hydroxylase of the pineal of *p*CPA-treated rats is much higher than the concentration of L-tryptophan. After loading of a large amount of L-tryptophan, the inhibition was overcome, resulting in an increase of serotonin in the pineal of *p*CPA-treated rats.

The observations reported here suggest that the biochemical mechanism of the depletion of serotonin is different between the pineal and brain of rats. The nature of the enzyme might be different between pineal tryptophan hydroxylase and cerebral tryptophan hydroxylase.

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TAKEO DEGUCHI*
JACK BARCHAS†

Department of Psychiatry,
Stanford University School of Medicine,
Stanford, California 94305

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* Present address: Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20014.

† Reprint requests to J. B.

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Toxicity of the Taste Testing Compound Phenylthiocarbamide

THE genetic polymorphism in human taste acuity for phenylthiocarbamide (phenylthiourea, PTC) and related compounds is widely used in practical demonstrations at most educational levels. The Nuffield O-level biology text¹ advises that PTC papers be prepared from 0.13% PTC solution, but PTC solutions or crystals are often used to distinguish "tasters" and "non-tasters".

In fuller investigations of the taste polymorphism, the technique devised by Harris and Kalmus², using a range of thirteen PTC solutions prepared by serial dilution by half of a 0.13% stock solution of PTC, is normal practice. The subject tastes successively stronger solutions of PTC until his taste threshold is reached. The tasting ability is then checked by an eight-cup sorting test in which the subject must distinguish cups of PTC from water. At least two sorting tests are required per subject. Experience has shown that most subjects swallow the test solutions, even when facilities for spitting out are available. It follows that the amount of PTC consumed depends on the taste acuity of the subject. A sensitive taster encounters only very dilute solutions, while an extreme non-taster can consume a considerable amount of PTC.

PTC tasting in schoolchildren was recently surveyed by P. E. J. W. using this technique. It was discovered afterwards that earlier workers had found PTC to be highly toxic to rats³⁻⁶, but this work is seldom quoted.

Richter and Clisby^{3,4} showed that a dose of 1.0 mg PTC could be fatal to laboratory rats, and Dieke *et al.*⁶ estimated the LD₅₀ for albino rats to be 3.1 ± 0.7 mg/kg. It was therefore decided to calculate the maximum dose that could be received by human subjects during taste testing. This is difficult to determine, as there is no limit to the volume of solutions used in the sorting test, and the cups may be refilled². We decided, however, to assume 10 ml./mouthful, as a small scale survey of eight subjects had an average mouthful size of 9.7 ml. The dosage of PTC per mouthful of each dilution was calculated, and from this the total maximum dose was worked out. On the expectation that a non-taster subject could consume 10. ml of each of thirteen dilutions, plus 40 ml. of each of the two most concentrated solutions, the total would be 104 mg

Table 1 Results of LD₅₀ Determinations for PTC in Rats and Mice

	Dose (mg/kg)	No. of animals per dose	% Mortality
Rat	2.0	9	33.3
	3.0	9	55.6
	4.0	10	60.0
	5.0	10	70.0
	6.0	9	100.0
Mouse	10.0	10	0.0
	20.0	10	0.0
	30.0	10	20.0
	40.0	10	60.0
	50.0	10	90.0

of PTC. This gives a dose of approximately 2 mg/kg for a 50 kg subject and 1.5 mg/kg for a 70 kg subject. These doses seemed undesirably close to the toxic level in rats previously quoted.

The acute toxicity of PTC in rats, and also mice, was determined. The PTC was administered to the animals orally by stomach tube, the animals having been fasted overnight. Owing to the low solubility of PTC in water it was prepared as suspensions of the appropriate concentration in gum acacia. Laboratory reagent grade PTC was used, as this would be the grade most frequently used in tasting tests.

The LD₅₀ in male albino TO strain mice of 18–22 g was found to be 37.0 mg/kg, with 95% confidence limits 31.6–43.3 mg/kg. In male albino Wistar rats of 250–300 g, the LD₅₀ was 3.0 mg/kg, with confidence limits 2.3–3.9 mg/kg. The method of Litchfield and Wilcoxon⁷ was used in calculating the LD₅₀ results (Table 1).

All the treated animals showed some respiratory difficulties, the extent and duration of the symptoms being dependent on the dosage received. Both rats and mice began to exhibit this effect 2 h after dosage, and deaths occurred from 4 to 24 h in rats and from 8 to 50 h in mice. The surviving animals returned to apparent normality over a period of 2 or 3 days. It was possible to confirm earlier reports^{3,4} of pulmonary oedema and pleural effusion in rats. Post-mortem examination revealed 3–6 ml. of fluid in the pleural cavities of the dead rats.

Our result of 3.0 mg/kg for the LD₅₀ of PTC in rats is in good agreement with that of Dieke *et al.*⁶ previously quoted. The oral LD₅₀ for PTC in rabbits is 40 mg/kg, while hens show an LD₅₀ of approximately 1,000 mg/kg⁸. There have been no reports of toxic effects of PTC in man, though Hutt⁹ reported vomiting after ingestion of PTC by two members of a family. It seems probable that there are species differences in susceptibility to PTC.

More recently it has been suggested that the toxic effects of PTC may result from desulphuration of the compound to liberate hydrogen sulphide in tissues. Scheline *et al.*⁸ used ¹⁴C and ³⁵S labelled PTC to demonstrate that the sulphur followed a different pathway from the remainder of the molecule. It has also been shown that thiourea derivatives which do not readily liberate hydrogen sulphide are relatively non-toxic¹⁰, whereas intravenous or intraperitoneal injections of aqueous hydrogen sulphide were fatal to rats within seconds¹¹. Hydrogen sulphide is highly toxic to man, producing among other reactions pulmonary oedema¹², indicating that the release of hydrogen sulphide from PTC could cause toxic effects if this reaction should occur in man.

Another thiourea derivative, α -naphthyl-thiourea (ANTU), has been used as a rodenticide, although this has now been withdrawn from use owing to the carcinogenic effects of free α -naphthylamine impurities. Norway rats, mice and dogs were most sensitive to ANTU, while guinea-pigs, cats, monkeys and chickens were more resistant. The LD₅₀ for oral dosage of ANTU in monkeys was found to be 4,250 mg/kg. The pulmonary oedema reaction was only found in Norway rats, dogs, mice and cats¹³.

PTC tasting is a valuable tool of the anthropologist, and it would be unfortunate if this test were to be abandoned owing to

fears of toxicity. Yet we must express our concern at the uncontrolled use of PTC in taste testing. The possible toxic effects of PTC should, we feel, be made known, even though PTC may eventually prove to be non-toxic to man. Although the doses used in class demonstrations of taste differences are very small it is necessary to consider the possibility of practical jokes and acts of bravado.

A further point concerns the possibility of long term toxicity effects of PTC. Richter and Clisby⁴ found that chronic dosage of PTC over a period of 6 weeks produced various effects in rats including hyperplasia and hyperactivity of the thyroid gland, atrophy of thymus and ovaries, and a decrease in body temperature and weight. In black rats there is a notable colour change, the fur turning grey³. It seems probable that PTC would act as a human goitrogen if taken in regular dosage; both thiourea and thiouracil have been used for this purpose in medicine, despite unpleasant and occasionally fatal side effects¹⁴.

As little is known of the possible effects of PTC in man, a full investigation of the problem is required. As an interim measure, it might be considered advisable to observe certain precautions in the use of PTC. For example, subjects performing tests should be advised to spit out the test solutions, as in wine tasting, and repeated testing of individuals should be avoided. In general we feel that it is important that the possible toxic effects of PTC are explained to potential subjects and research workers before they embark on any taste testing.

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PENELOPE E. J. WHEATCROFT
C. C. THORNBURN

Department of Biological Sciences,
University of Aston in Birmingham,
Gosta Green,
Birmingham B4 7ET

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Effects of Gonadotrophins on Motility of Human Ovary

THE occurrence of smooth muscle cells in the ovaries of many mammalian species suggests their possible function in the rupture of the ripe follicle by their contractions¹. In the rabbit, pig and sheep ovarian follicles, true smooth muscle cells are present in either or both the theca interna and externa. In women, follicles are surrounded by a network of reticular fibres and cells which resemble smooth muscle cells without